**Advances in male sterility system: Genetic & molecular concepts and breeding strategies**

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**Introduction**

The world's population is expected to **increase by 25%** by the middle of the twenty-first century, reaching 10 billion people. It represents an enormous risk to global food security. Due to the limited amount of freshwater and agricultural land, modern agricultural technologies must be used to produce more sustainably. Global crop production has significantly benefited economically from the use of heterosis. Hybrid cultivars account for more than half of major crops such as rapeseed, sunflower, sorghum, maize and rice. As a result, hybrid breeding makes significant contributions to the global food supply. Rice, pearl millet, sorghum, maize, barley and rapeseed are making incredible contributions to the hybrid breeding system. The characteristics seen in hybrid plants include increased uniformity, improved abiotic and biotic stress tolerance and better adaptability.

A well-known condition in higher plants known as **male sterility**, is a situation where the male reproductive parts of a plant are either absent, aborted or nonfunctional and hence they fail to participate in the process of natural sexual reproduction (Saxena and Hingane, 2015). Male sterility in plant is considered as typical example of maternal inheritance because it is transmitted through female only. It is caused by barrier of tapetal layer, improper timing of callase activity, abnormal micro-sporogenesis, involvement of esterase, absence or malformation of male organs (stamens). The first-person Joseph Gottlieb Kolreuter (1763) who record the presence of plants in nature with damaged anthers in specific natural populations. Later, Jones & Emsweller (1937) and Stephens & Holland (1937) proved male sterility for the development of hybrid onion and sorghum seeds, respectively. Any irregularities observed in development at any stage of pollen grain or microsporogenesis release may give rise to disability. In addition to cytoplasmic male sterility (CMS) and genetic male sterility (GMS), it is present in about 610 plant species. CMS is caused by the interaction of genes in the cytoplasm and nucleus, whereas GMS is solely controlled by nuclear genes. CMS are reports of it in over 200 plant species. Effective use of CMS has made easier to incorporate the desired characters into hybrids. Male-sterile plants provide crucial breeding tools to harness hybrid vigor or heterosis, in hybrid crops and also provide essential materials to study stamen and pollen development and cytoplasmic-nuclear genomic interactions (Chen and Liu, 2014).

CMS is associated with unusual **open reading frames** (ORFs) found in mitochondrial genomes (Chase and Gabay-Laughnan, 2004; Hanson and Bentolila, 2004). Nuclear encoded genes which restored the male fertility, termed as **restorer-of-fertility (*Rf*)** factor, have the significant agronomic benefit for producing hybrid seeds, frequently used in crop production. Fertility is restored by a series of *Rf* genes (*Rf1*, *Rf1a*, *Rf1b*, *Rf2*, *Rf3*, *Rf4*, *Rf5*, *Rf6, Rf17 etc.*) encoded in the nucleus. Total nine *Rf* genes have been isolated in seven plant species *like*., maize, *Petunia*, radish, *Brassica*, rice, sorghum, sugar beet *etc*. First isolated restorer gene in **CMS-T maize is *Rf2***.

Most of the genes encode specific **PPR** (pentatricopeptide repeat) proteins involved in processing of mitochondrial mRNA. For instance, pentatricopeptide repeat (PPR)-containing proteins are around 450 and 650 nucleus-encoded in *Arabidopsis* and rice, respectively. Most PPR proteins target the mitochondria or plastids for their activities. Male sterility results from mitochondrial genes causing cytoplasmic dysfunction, and fertility restoration relies on nuclear genes that suppress cytoplasmic dysfunction (Eckardt, 2006).

**Three-line breeding system/CMS system**

CMS based hybrid seed technology involves the CMS Lines (A line), Maintainers lines (B line) and Restorer lines (R line). Three lines are involved so it is known as **Three-line breeding system**. The line which has male-sterile cytoplasm with CMS-causing gene and absence of functional restorer of fertility genes (*Rf*), that female parent is called as CMS lines. The line having normal fertile cytoplasm but similar nuclear genome *like*, CMS line and used as male parent in crossing for the propagation of the CMS lines is termed as maintainer lines. The restorer line functional *Rf* genes used as male parent to produce F1 hybrids crossing with the CMS lines. *Rf* genes have ability to restores male fertility in the F1 hybrids (**Figure 1**).

 **Figure 1: Three-line breeding system**

**Association of mitochondrial genes in CMS**

CMS and fertility restoration **reveal novel perspectives on the molecular genetic** relationships between plant mitochondria and the nucleus. Plant mitochondrial genes that determine CMS are analysed in the context of genomic characteristics that likely have a role in origin and spread of these genes. The molecular and cellular studies of CMS phenotypes, as well as the molecular studies of restorer genes, are presented, along with the insights gained from these studies into signalling pathways and the role of nuclear genes in plant mitochondrial gene expression.

Numerous metabolic pathways are carried out by mitochondria that are essential to higher eukaryotic life. It includes the pathways *like.*, tricarboxylic acid cycle, respiratory electron transfer and ATP synthesis. Spans of DNA sequences or stretches of DNA sequence between start and stop codon called as **open reading frame (ORFs)**. It has the potential to be transcribed into RNA and translated into a protein. So, it requires a continuous sequence of DNA from start codon then multiple of three nucleotides to a stop codon in same reading frame. Open refers to the fact that the **road is open** for ribosome to read continuously triplet after triplet untilribosome meets this stop codon.

Minimum **14 mitochondrial genes** that determine CMS have been characterized as open reading frames (ORFs) comprising segments derived from mitochondrial gene-coding and gene-flanking sequences and from sequences of unknown origin (Chase and Gabay-Laughnan, 2004; Hanson and Bentolila, 2004). ***Cox1, atp8* and *atp6*** mitochondrial genes are frequently involved in the origination of CMS genes. Recombination occurs in genome of mitochondria which is responsible for the formation of ORFs and their placement downstream of sequences that support gene expression. **ORFs are expressed** because **they are fused directly to mitochondrial promoter sequences** or are co-transcribed with upstream mitochondrial genes (Chase, 2007).

**CMS associated genes in various crops species**

Several CMS cytoplasm *were* recovered from the breeding lines. Many putative mitochondrial ORFs have been found in rice. CMS-WA and CMS-RT102, with *WA352* and its variant *orf352,* respectively, consist of *orf284, orf224*, *orf288* three segments in the mitochondrial ORFs and a short sequence of unknown origin (SUO). CMS-CW with CW-*orf307* include two segments: *orf288* and a SUO. CMS-BT with *orf79* (derived from the indica rice variety Boro II) and its variant CMS-HL with *orfH79* (derived from the wild rice accession Hong-Lian) encode small proteins B-*atp6* at N terminus similar with the *cox1* and the remaining sequence of unknown origin (SUO) portion. In Sorghum, CMS-A3 is associated with *orf107*, whichencodes *atp9* protein at the N terminus and other remaining portions, similar to rice *orf79*. In Wheat, CMS-AP line having the *Triticum aestivum* nuclear genome and *Triticum timopheevii* cytoplasm, is associated with *orf256*. The 5' region of *orf256* and the first 11-amino-acid coding sequence are equally similar to *cox1*.

In the dicot species, the CMS genes are presented with *atp8* sequences. The *Brassica* CMS-Ogu and CMS-Kos genes, *orf138* and *orf125*, which originated from radish, encode *atp8-like* proteins. The *Brassica* CMS-Pol and CMS-Nap genes, *orf224* and *orf222*, encoding membrane proteins with 79% sequence similarity, contain an *atp8*-derived sequence and a SUO (Singh and Brown, 1991). The *atp8* sequences are also present in *orf522* in sunflower CMS-PET1 and *orfB-cms* in carrot CMS-Petaloid, with an additional SUO. Many other CMS genes contain *atp6* sequences of different lengths, including *atp6*-C in maize CMS-C and *preSatp6* in sugar beet CMS-Owen. So, many recognized CMS genes (*like*, *orf125* and *orf138* in radish with CMS-Kos and its variants *Brassica* with CMS-Ogu, respectively.) and *cox2* mutated in CMS-G of sugar beet are nonchimeric genes that include sequences from single sources **(Table 1)**.

**Table 1: Different crop species with CMS lines and their associated genes**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sr. No** | **Crop species** | **CMS line** | **Associated genes** |
| 1 | Brassica | CMS-Ogu | *orf138* binds with *atp8-like* proteins. |
| CMS-Pol | *orf224* with 2 segments *like*, *atp8* & short sequence of unknown origin (SUO). |
| CMS-Nap | *orf222* similar with *orf224.* |
| 2 | Carrot | CMS-Petaloid | *orfB-cms* with *atp8* andSUO. |
| 3 | Maize | CMS-T | *orf221* with *atp4* and 5’ UTR having *atp6* encodes with *urf13.* |
| CMS-S | *orf355* and *orf77* with 2 segments of *atp4* & *atp6* and both ORFs combinedly known R sequence. |
| CMS-C | *atp6-*Cinvolving the short sequence of unknown origin (SUO) and *atp6.* |
| 4 | Rice  | CMS-WA | WA352 with *orf284, orf224*, *orf288* three segments in ORFs and a SUO. |
| CMS-RT102 | *orf352* is variant of WA352. |
| CMS-CW | *CW-orf307* having *orf288* and SUO. |
| CMS-BT | *orf79* encode small proteins B-*atp6* at N terminus similar with the *cox1* and the remaining SUO. |
| CMS-HL | *orfH79* is similar with *orf79.* |
| 5 | Radish | CMS-Don | *orf463* binds with *cox1* and SUO. |
| CMS-Kos | *orf125* binds with *atp8-like* proteins. |
| 6 | Sorghum | CMS-A3 | *orf107* with *atp9* protein at the N terminus and other remaining portions just similar to rice *orf79*. |
| 7 | Sugar beet | CMS-Owen | *preSatp6* encodes with *Satp6.* |
| I-12CMS (3) | *orf129* with *cox2* and SUO. |
| 8 | Sunflower | CMS-Baso/PET1 | *orf522* with involving *atp8,* SUO & *atp9* segments. |
| 9 | Wheat  | CMS-AP | *orf256* encodes the *cox1* |

**Genetic and molecular basis of male sterility**

The various models explained the sterility that exhibits in plant, the important models are discussed below:

**1) The cytotoxicity model:**

Contains CMS proteins, which cause immediately **cell death** in sterile plants. The cytotoxicity of CMS proteins was evaluated on the bases of transgenic expression of CMS genes in prokaryotic and eukaryotic cultured cell systems. The CMS protein normally has a molecular weight between 10 and 35 kDa with transmembrane proteins and contains hydrophobic regions, which are characteristics of cytotoxic proteins. In very simple term, CMS proteins cause mitochondrial dysfunction in the saprophytic or gametophytic cells of the anther which cause male abortion (Levings, 1993). URF13, the first CMS protein found in maize CMS-T, is harmful to many eukaryotic cells as well as *Escherichia coli* (Korth et al., 1991; Korth and Levings, 1993).

**2) The energy deficiency model:**

Mitochondrial electron transfer chain, or mtETC, is the mechanism by which plant cells produce biological energy (ATP) during **respiration**. Research conducted on the sporophytic and gametophytic cells of plant anthers revealed that they have a **higher energy** required than different parts of the plant. The cells are **unable to meet the energy requirements** of the development of the male reproductive organs due to the difficulties caused by CMS proteins, according to this model. For the energy deficiency model, CMS protein structure has sufficiently **supplied the molecular base**. Numerous CMS proteins, including *preSatp6* of CMS-Owen in sugar beet, *orf138* of CMS-Ogu in Brassica, *orf79* of CMS-BT in rice, *orfH78* of CMS-HL in rice and *URF13* of CMS-T in maize, are mitochondrial transmembrane proteins. These proteins are combine to the inner membrane of the mitochondria, disrupting the proton gradient and having an impact on the generation of ATP (Rhoads *et al.,* 1995). CMS genes involve some **necessary mitochondrial genes** which are involve in respiration process *like.*, *nad3*, *nad5* and *nad7* for complex I; *cox1* and *cox2* for complex IV; and *atp1*, *atp4*, *atp6*, *atp8* and *atp9* for complex V. CMS proteins engage in competitive interactions with mtETC. CMS genes strongly indicates the connection between **respiratory pathways and CMS**.

**3) The aberrant programmed cell death model:**

**Apoptosis** *is a cellular process involving nuclear DNA fragmentation* and is controlled by mitochondrial-derived signals. PCD includes the germination of seeds, elongation of root tips, senescence, organ development and more. The **cytochrome *c*** is released from mitochondria into the cytosol which has the major role in the plant PCD (Liu *et al*., 1996). The **interaction between the sporophytic and gametophytic cells** must occur for the formation of male gametophytes in anthers and also required controlled PCD on cellular degradation of the tapetum, the inner most cell layer of anther wall tissue (Ma, H., 2005). As a result, function of normal tapetum needed the timely initiation & progression of PCD and **premature or delayed which leads to the male sterility** (Ji *et al.,* 2013). For example, in sunflower CMS-PET1 cytoplasm, the premature PCD of the tapetal cells is observed, and it is also associated with releases of cytochrome *c* from mitochondria to the cytosol (Balk and Leaver, 2001).

**4) The retrograde regulation model:**

Retrograde means reverse, this regulation is the general term used for mitochondrial signalling. During retrograde signalling, **they are sent to the nucleus instead of signals leaving the nucleus**. Transcription factors **MADS box** are involved in all major aspects related to the male and female gametophyte development, embryo and seed development, and root, flower, and fruit development. MADS box is **supressed in the CMS lines**. For example, MADS-box genes expression in carrot flower controlling the whorls 2 and 3 is suppressed in the carpeloid CMS lines. This strongly indicates that retrograde signalling from mitochondria regulates the expression of these nuclear MADS-box genes, **determining the organ conversion** in carpeloid CMS (Linke *et al.*, 2003). Increased RMS (Retrograde-regulated male sterility) expression suppresses pollen germination, thus leading to male sterility.

**Fertility Restoration**

The expression related with CMS genes can be suppressed or counteracted by the products of specific restorer genes, thereby allowing pollen fertility. Fertility-restoring alleles are giving information related to genetic crosses involving a male-sterile seed parent and a restorer parent of a different nuclear genotype. Restoration pattern is divided into two parts: **sporophytic (anther wall) and gametophytic (microspore)** restorer. In which sporophytic restorer carried out in either sporophytic tissue or before the meiosis. Gametophytic restorer is done after meiosis in microspores or pollen grains. Both follow the **different transmission pattern**.

A diploid plant having a male-sterile cytoplasm and being heterozygous in nature for a restorer gives rise to two different pollen grain classes: those that carry the restorer and those that do not. Both types of genotypic classes of gametes will be functional in the case of **sporophytic restorers.** A plant with a heterozygous nature for a **gametophytic restorer** that only gametes carry the restorer allele will be functional. For example, Sorghum fertility-restoring genes *Rf1* and *Rf2* for CMS A1 (Klein *et al.,* 2005; Jordan *et al*., 2010), *Rf5* (Jordan *et al*., 2011) and *Rf6* (Praveen *et al*., 2015) for CMS A2 are **sporophytic** fertility restorer genes, and *Rf3* and *Rf4* function as **gametophytic**-restorers for CMS A3. Another well-known example is that the S-cytoplasm of maize is characterized as a CMS system that is restored gametophytically.

**Why restoration is essential?**

* Cytoplasmic male sterility is a maternally inherited trait often associated with the **mitochondrial genome.**
* Male sterility alone does not fulfill the purposeof hybrid seed production as it should be overcome in the F1 hybrid generation to achieve the goal of hybrid seed production**.**
* In cells, the nuclear genes called restorer of **fertility (*Rf*) can restore pollen fertility.**
* At the time of pollen development,**the *Rf*** **genes block or compensate for mitochondrial dysfunctions** that are phenotypically expressed. Thus, for a **successful hybrid seed production** program, the **need for restorer of fertility (*Rf*)** is important as the male sterility system will not serve the purpose.

**Genetic basis of fertility restoration**

The diversity in the restoration pattern increases **the number of restorer genes**. Generally, one or two major restorer loci is completely involved in the restoration pattern. Sometimes full restoration needs the **jointly planned** action of number of genes, in which some provide only **small or minor effects on restoration**. For Instance, Single gene was responsible for fertility restoration of A1 male-sterile cytoplasm of sorghum (Murthy and Gangadhar, 1990). In other hand, in T-cytoplasm maize, PET-cytoplasm sunflower and T-cytoplasm onion, two unlinked restorers are required for full restoration (Schnable and Wise, 1998). Duplicate restorer loci available in many numbers of systems. In maize, T-cytoplasm *Rf1* is major restorer with partially substitute *Rf8* gene*.* Same cases also available in sunflower with PET1 cytoplasm, onion with T-cytoplasm and *Phaseolus* with CMS. Such overlapping functions could result from duplication of gene functions or indicate that multiple mechanisms can induce restoration.

**The first *Rf2* restorer gene was found in maize**. Male fertility restoration in CMS-T type, restorer allele *Rf2* is required, with a combination of *Rf1* restorer allele. (which is not linked with *Rf2* gene). *Rf2* encodes a functional mitochondrial aldehyde dehydrogenase, exhibit that fertility restoration through metabolic compensation for the effects of a mitochondrial CMS-determining gene. Different crop species *like*., Maize, rice, sunflower, brassica, radish, sorghum, wheat, common bean, carrot, sugar beet *etc*. available CMS systems and their *Rf* genes mentioned below **(Table 2)**.

A series of *Rf* genes are encoded by the pentatricopeptide-repeat (PPR) protein family members. PPR Proteins is the largest protein families in the earth plants. It is key regulatory of the plant mitochondrial gene expression. It is generally found in eukaryotes. It is present in either mitochondria or chloroplast, where they modulate gene expression at the RNA level. PPR motifs are degenerate motifs, each with 35-amino-acid sequences and are present in tandem arrays of 2-27 repeats per protein (Saha *et al.*, 2007) **(Figure 2)**. Different functional-related studies on PPR proteins have revealed their role in RNA processing, embryogenesis, fertility restoration in CMS plants and plant development.

**COO‑**

**NH2**

**Variable length organelle targeting sequence**

**2-27 PPR repeats in tandem 35 amino acid per repeat unit**

**C terminal optional motif**

**Figure 2: PPR protein structure arrangement**

**Table 2: Cytoplasmic male sterility (CMS)/restorer (*Rf*) gene systems in major crops**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Crop species** | **CMS type** | ***Rf* locus** | **Protein property** | **References** |
| Brassica (*B. napus*) | CMS-Ogu (S) | *Rfo* (P) | PPR Protein | Brown *et al*., 2003Uyttewaal *et al*., 2008 |
| CMS-Pol (S) | *Rfp* (R) | unknown | Singh and Brown., 1991 |
| CMS-Nap (S) | *Rfn* (R) | unknown | L’Homme *et al*., 1997 |
| Brassica (*B. juncea*) | CMS-Hau (S) | *UK* | unknown | Jing *et al*., 2012 |
| CMS-orf220 | *UK* | unknown | Yang *et al.,* 2010 |
| Brassica (*B. tournefortii*) | CMS-Tour (S) | *Unknown* (P) | unknown | Landgren *et al.*, 1996 |
| Carrot (*Daucus carota*) | CMS-Petaloid | *Unknown* (R) | unknown | Nakajima *et al.*, 2001 |
| Common bean (*Proteus vulgaris*) | CMS-Sprite(S) | *Fr* (G)*, Fr2* (P) | unknown | Abad *et al.*, 1995 |
| Maize (*Zea mays*) | CMS-T (S) | *Rf1* (R) | unknown | Dill *et al*., 1997 |
| CMS-S (G) | *Rf2* (M) | unknown | Zabala *et al*., 1997 |
| CMS-C (S) | *Rf3* (R) | unknown | Dewey *et al.*, 1991 |
| Radish (*Raphanus sativus*) | CMS-Kos (S) | *Rfk1* (P) | PPR Protein | Iwabuchi *et al.,* 1999 |
| CMS-Don (S) | *Rfd1* (P) | unknown | Park *et al*., 2013 |
| Rice (*Oryza sativa*) | CMS-BT (G) | *Rf1a* (R)*, Rf1b* (R) | PPR Protein | Akagi *et al.*, 2004 Komori *et al*., 2004Wang *et al*., 2006 |
| CMS-HL (G) | *Rf5 (Rf1a)* (R) | PPR Protein | Wang *et al*., 2013 |
| CMS-LD (G) | *Rf2* (P) | Glycine-rich protein | Itabashi *et al*., 2011 Itabashi *et al*., 2009 |
| CMS-CW (G) | *Rf17* (P) | Acyl-carrier protein synthase | Fujii and Toriyama, 2009 |
| CMS-WA (S) | *Rf3* (P)*, Rf4* (R) | unknown | Zhang *et al*., 1997 Zhang *et al*., 2002 |
| CMS-RT120 | *Rf102* | unknown | Okazaki *et al*., 2013 |
| CMS-RT98 | *Unknown* | unknown | Igarashi *et al*., 2013 |
| Sorghum (*Sorghum bicolor*) | CMS-A3 (G) | *Rf3* (R) | unknown | Tang *et al*., 1996 |
| CMS-A1 (G) | *Rf1, Rf2* | PPR protein | Klein *et al.,* 2005 |
| Sugar beet (*Beta vulgaris*) | CMS-Owen | *Rf1* (P) | Peptide | Matsuhira *et al*., 2012 |
| I-12 CMS-(3) | *UK*(P) | unknown | Yamamoto *et al*., 2008 |
| CMS-G | *RfG1*, *RfG2* | unknown | Ducos *et al.*, 2001 |
| Sunflower (*Helianthus annuus*) | CMS-PET1 (G) | *Rf1* (R) | unknown | Horn *et al.,* 2003 |
| Wheat (*Triticum aestivum*) | CMS-AP | *Unknown* | unknown | Song and Hedgcoth, 1994 |

**Mechanism of fertility restoration**

CMS protein cause the CMS in plants. At the time of pollen development,the *Rf* genes block or compensate for mitochondrial dysfunctions that are phenotypically expressed. Fertility restoration process involve the mitochondrial nuclear gene interaction. **(Figure 3)**. Mitochondrial-sorting gene (MSG) products with RF proteins and tissue-specific regulatory factors (TSRFs) are encoded in the nucleus and target the mitochondria for anterograde regulation by subunits of the mitochondrial electron transfer chain (mtETC) complexes. The translational or posttranslational level may be regulated by tissue-specific regulatory factors, the male organ-specific accumulation of CMS proteins for male specificity. The CMS proteins and mtETC subunits interact with each other and affect their activities or ATP synthesis, producing retrograde signals that trigger aberrant programmed cell death (PCD) in tapeta or microspores. Restoration of CMS by RF proteins can be achieved at the genomic level, posttranscriptional level, translational or posttranslational level and metabolic level.



**Figure 3: Generallized mechanism of CMS/*Rf* System (Modified from Chen and Liu, 2014)**

1. **Restoration at Genomic Level**

The genome of the mitochondria is **highly dynamic** and shows **frequent variations** in the structure and copy number of mitochondrial DNA molecules. In some CMS plants, spontaneous reversion of fertility occurs, and **sub-stoichiometric shifting** involves the relative copy numbers of certain sub-genomic molecules containing CMS genes. Pollen fertility restoration may be accompanied by the loss**of an mt-DNA fragment from the mitochondrial genome.** For instance, mitochondrial PVS sequence is caused by **CMS Sprite in common bean**. It is the **first example** of *Rf* gene restoration at the genomic level through sub-stoichiometric shifting. The presence of the dominant nuclear gene *Fr* which produced cut in the PVS mitochondrial genomic sequence. So, CMS-associated mitochondrial DNA molecules convert in the normal state and here, the nuclear gene *Fr* is present in the progeny of the subsequent generation which means generation is fertile (Xu *et al*., 2022)**.**

1. **Restoration at the** **Posttranscriptional Level**

Expression and sequencing analyses have provided information regarding most CMS-associated transcripts in crop species. CMS-associated transcripts are processed using *Rf* gene products. ***Rf* proteins suppress CMS gene expression** through posttranscriptional mechanisms such as editing, splicing, and cleavage. In the RNA editing process, **cytidine (C) residues change to uridine (U) at certain sites of the RNA sequences** in plant organelles, especially in mitochondria. RNA exo/endonucleolytic cleavage may occur in multicistronic transcripts' coding regions and/or the intercistronic (spacer) sequences. For example, Four C-to-U editing sites are present in the *orf107* of **CMS-A3 of sorghum**. If the plant is sterile than site 1 and 2 edited frequently and infrequently respectively. *Rf3* gene is required in the action of site 3 and 4 approximately 80% and 60% effectiveness. *orf107* is degraded in the plants which has *Rf3* is present (Tang *et al*., 1999). In **CMS-T of maize** restore the fertility by *Rf1* and *Rf2* gene. CMS-T is associated with *urf13-orf221* dicistronic transcript is processed with *Rf1* and reduced the abundance of cleaved *urf13* RNA fragment (Kennell and Pring, 1989). In **CMS-BT rice**, cleaves the *B-atp6-orf79* dicistronic transcripts by *Rf1a* and *B-atp6* is available at 5' untranslated region, but in some case *B-atp6-orf79* transcripts is degraded by *Rf1b*. If the restorer plant has presence of *Rf1a* and *Rf1b* than *Rf1a* gave the information about epistatic effect on the cleavage of the transcripts. The cleaved *orf79* RNA fragment lost from its ribosome-binding site and is not translated (Wang *et al.*, 2006). Similar process is happened in the *atp6-orfH79* of **CMS-HL** (Yi *et al*., 2002). *Rf1a* of CMS-BT and similar gene *Rf5* ofCMS-HL observed the similar kinds of cleavage pattern, but difference is that *Rf1a* is directly binding to the *B-atp6-orf79* mRNA (Kazama *et al.*, 2008) and *Rf5* required glycine-rich protein *GRP162* (used as adaptor in restoration of fertility complexes) which is bind to the *atp6-orfH79* mRNA for cleavage (Hu *et al.*, 2012). *Rf4* of **CMS-WA** degrade the *rpl5-WA352* dicistronic transcripts and *WA352* monocistronic transcripts and also reduced the abundance of this transcripts nearly 20% in the *Rf4*-restored plants (Luo *et al*., 2013).

1. **Restoration at the Translational or Posttranslational Level**

In some crop plant species CMS systems, the **size and amounts of CMS-associated transcripts do not change.** At that time, translational or posttranslational fertility restoration mechanisms occurs. For example, *Rf4* of CMS-C in **maize** does not change the steady-state level of *atp6-C* mRNA, so we know that restoration may present at the protein level (Dewey *et al*., 1991). *Fr2* of CMS-Sprite in common bean does not affect the PVS transcript but overwhelms the accumulation of *orf239* protein (Sarria *et al*., 1998).CMS-*Ogu* of **brassica and radish** which is restored by *Rfo* and encodes the PPR-B (also named *Orf687*). The amount of *orf138* mRNA amount is not altered, but suppression of accumulated *orf138* protein. PPR-B and *orf138* mRNA bind together, most of the time blocking the *orf138* translation (Uyttewaal *et al.*, 2008).

1. **Restoration at the Metabolic Level**

Some *Rf* gene encodes for the production of enzymes. This **enzyme converts harmful molecules into non-harmful molecules** thus, thus restoring fertility by eliminating the harmful effects of such harmful molecules. For example, *Rf2* of **CMS-T in maize** which is encodes an aldehyde dehydrogenase enzyme. It plays important roles in the metabolism of fatty acids & amino acids and detoxify alcohols and toxins by altering aldehyde damage to cells and tissues. Oxidization is done by RF2 protein at least three aldehydes. Given that neither the *urf13-orf221* transcripts nor the *URF13* protein is changed in the presence of *Rf2*, *RF2* may restore CMS-T by eliminating harmful molecules caused by *URF13.* (Liu *et al*., 2001).

**Conversion of agronomically ideal genotypes into male sterile: concepts and breeding strategies**

**Attributes of Agronomically Ideal Genotypes**

The term agronomically ideal genotype refers to a plant variety or cultivar that exhibits specific characteristics and traits optimized for increasing production, efficiency and sustainability in agriculture (Khush, 1999). In addition to pests, diseases, environmental stresses and market demands, these genotypes are converted to meet the needs of farmers (Tester and Langridge, 2010). Depending on the crop species, growing conditions and specific breeding objectives, agronomically ideal genotypes may have different characteristics. However, some common attributes include:

* **Yield Potential:** Agronomically ideal genotypes typically produce high yields when grown under optimal conditions. Farmers rely on this trait to maximize production and profitability.
* **Disease Resistance:** To reduce crop losses and minimize pesticide use, crops must resist pathogens such as fungi, bacteria, viruses and nematodes.
* **Pest Resistance:** Crops with pest resistance are protected from damage and require fewer insecticides to thrive, making them environmentally sustainable.
* **Abiotic Stress Tolerance:** It might be resistant to several abiotic factors, including heat, salinity, cold and drought, ensuring constant yields even in unfavourable environmental conditions.
* **Adaptability:** Adaptability to diverse agro-climatic conditions and cropping systems enables farmers to grow these genotypes across different regions and environments.
* **Quality Traits:** Quality attributes such as nutritional content, taste, texture and shelf-life are important for meeting consumer preferences and market demands.
* **Efficient Resource Use:** Genotypes that utilize water, nutrients and other resources efficiently contribute to sustainable agriculture by reducing resource wastage and environmental impact.
* **Early Maturity:** Early maturing genotypes allow for shorter cropping cycles, enabling farmers to grow multiple crops within a single growing season or to adapt to shorter growing seasons.
* **Uniformity and Stability:** Uniformity in traits across plants and stability in performance over different seasons and environments are desirable characteristics for ensuring consistent and reliable crop production.
* **Ease of Management:** Genotypes that are easy to manage, harvest and process contribute to labor efficiency and cost-effectiveness for farmers.

**Challenges in introducing male sterility into agronomically ideal genotypes**

Introducing male sterility into agronomically ideal genotypes presents several challenges due to the complex genetics and physiological mechanisms involved (Varshney *et al.,* 2012). Male sterility is often utilized in hybrid seed production, which facilitates the production of high-yielding hybrid cultivars. However, incorporating male sterility into ideal genotypes can be challenging due to issues such as genetic instability, linkage drag, and difficulty maintaining agronomic performance. Here are some challenges described below:

* **Genetic Stability and Segregation:** Maintaining genetic stability while introducing male sterility genes into agronomically ideal genotypes is crucial to avoid unintended changes in important agronomic traits. Ensuring proper segregation of male sterility traits in subsequent generations is essential for stable performance.
* **Linkage Drag:** Male sterility genes may be linked with undesirable traits, leading to linkage drag and compromising the overall agronomic performance of the genotype. Overcoming linkage drag requires precise genetic manipulation and selection strategies to uncouple male sterility from undesirable traits.
* **Maintaining Agronomic Performance:** Introducing male sterility should not compromise the overall agronomic performance of the genotype, including yield potential, stress tolerance and quality traits. Maintaining or even enhancing agronomic performance while incorporating male sterility requires careful selection and breeding strategies.
* **Environmental Sensitivity:** Male sterility may be sensitive to environmental factors such as temperature, photoperiod and nutrient availability, leading to variable expression and reduced efficiency under different growing conditions (Reynolds and Langridge, 2016). Developing male sterile lines with stable and robust performance across diverse environments is a significant challenge.
* **Transgene Flow and Biosafety Concerns:** Introducing male sterility through genetic modification raises concerns about transgene flow and potential environmental impacts. Addressing biosafety concerns and regulatory requirements is essential to ensure the safe deployment of male sterile genotypes in agricultural systems.

Overcoming these challenges requires interdisciplinary research efforts integrating genetics, genomics, breeding and biotechnology to develop male sterile genotypes with improved stability, performance and environmental safety.

**Concepts of Conversion into Male Sterile Genotypes**

Converting crops into male sterile genotypes is a critical step in hybrid seed production, enabling the efficient production of high-yielding hybrid cultivars. Several approaches have been developed to induce male sterility, including genetic modification, chemical treatments and cytoplasmic male sterility (CMS). Here are some concepts of conversion into male sterile genotypes:

1. **Genetic Modification:**

Genetic modification involves the insertion of male sterility genes or disrupting genes essential for pollen development using techniques such as CRISPR-Cas9. This approach allows precise manipulation of the plant genome to induce male sterility while maintaining agronomic performance.

By using sequence-specific nucleases, genome editing technology allows for extremely precise modifications to the host plant's genome, including only a few base pair alterations in the target gene sequence. When implemented properly, this technique produces transgene-free plants and minimizes many regulatory authorities' concerns about biosafety. Zinc Finger Nucleases (ZFN), TALE-fused nucleases (TALENs) and the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 system are some examples of genome editing techniques (Cong *et al*., 2013; Cox *et al*., 2015; Zhang *et al.,* 2013). Because CRISPR technology is simpler and less demanding technologically than the previous two technologies, researchers are more attracted to using it for gene/genome editing.

Technologies for editing genes and genomes can accurately improve genetic features and speed up breeding cycles by reducing breeding time. One of the many important advantages of using this technology is the production of male sterile plants. For example, Zhou *et al.* (2016) used CRISPR/Cas9-mediated knockout of the *TMS5* gene to create the "transgene-clean" thermo-sensitive genic male sterile (TGMS) rice lines (Zhou *et al.,* 2016). The fact that the genome-edited TGMS lines were produced in just one year shows how effective genome editing is at facilitating the breeding process. A similar method was utilized to knock out the ZmTMS5 gene, a homolog of rice TMS5, to generate maize TGMS lines (Li *et al.,* 2017). Li *et al*. (2016) used CRISPR/Cas9 to modify the carbon starved anther (CSA) gene to create a photoperiod sensitive genic male sterile (PGMS) japonica rice 9522 line. These genome-edited plants showed male sterility under short-day conditions (10.5 h photoperiod). However, partial male fertility was seen under long-day conditions (14 h photoperiod). On the other hand, it was also noted that CSA gene editing in the japonica variety Kongyu-131 demonstrated sensitivity to temperature and day length, suggesting that different alleles may influence rice plant fertility in response to particular environmental conditions in different genetic backgrounds.

Okada *et al.* (2019) used CRISPR/Cas9 to quickly knock out the fertility-related gene *Ms1* to produce male-sterile wheat lines for commercial use. Male sterility was generated in tomato plants using CRISPR/Cas9-targeted mutation of the stamen-specific gene *SlSTR1* (Du *et al*., 2020). In maize, Arabidopsis and rice, the *ZmMs7* gene was completely knocked out using CRISPR/Cas9, resulting in male sterility (An *et al.,* 2020). Mutations in pollen and embryo sacs in rice plants caused by a CRISPR/Cas9-mediated mutation in the *OsROS1* gene were noted by Xu *et al*. (2020). Zhang *et al.* (2020) used CRISPR/Cas9-edited alterations in ryegrass's *LpDMC1*, a crucial meiosis-related gene, to produce fully male-sterile plants. Male sterility was also caused by a CRISPR/Cas9-mediated mutation of the soybean homolog of *ABORTED MICROSPORES (AMS)* gene, *GmAMS1*, although *GmAMS2* did not cause male sterility. This suggests an important role of *GmAMS1* in tapetal development (Chen *et al*. 2021). The above examples demonstrate the technology's ability to quickly and easily create male sterile lines by deleting certain genes involved in anther development and fertility restoration, among other genes. Gene editing systems can edit multiple genes simultaneously by employing numerous domains in the vector to target many genes in a single operation. With this preference, the technique is ideal for creating stable enough materials to manipulate targets. As a result, the technology might start to be used by the researchers regularly. In this direction, Singh *et al.* (2018) showed that the CRISPR/Cas9 system allowed for the quick creation of male sterile bread wheat, *Triticum aestivum* L., through triple homozygous mutations of the *Ms45* gene. Li *et al.* (2020) employed a similar methodology to produce a triple mutant of the wheat *TaNP1* gene, which resulted in male sterility. Liu *et al.* (2022) used a different study to illustrate the multi-gene editing technique by introducing numerous mutations in homologous genes affecting maize male fertility and pollen maturation. The findings showed that total male sterility requires a triple homozygous gene mutation of *ZmTGA9-1/-2/-3*. However, double-gene mutants of ZmDFR1/2 and single-gene mutants of ZmACOS5-2 also displayed male sterility.

Additionally, the restoration of male fertility was demonstrated by eliminating genes linked to CMS. For example, male fertility was restored when orf79 in boro rice and orf125 in Kosena-type CMS of rapeseed were knocked out using mitochondria-targeted TALENs (Kazama *et al*., 2019). A similar method was also employed to eliminate the orf312 in rice Tadukantype CMS (TAA), restoring fertility (Takatsuka *et al*., 2022). The various systems developed through the genetic engineering approach are listed in **Table 3** and the phenomenon is depicted in **Figure 4.**



**Figure 4 Schematic depiction of genetically engineered male sterility and fertility restoration systems for hybrid variety development (Modified from Gautam *et al.,* 2023)**

Using a genetic modification technology, male sterile transgenic plants are created by introducing gene sequences exclusive to the male reproductive system that either prevent or disrupt pollen generation or anther development (microsporogenesis). As a result, only female (or "male-sterile") plants are produced, which can be maintained and utilized to make hybrid seeds. Because anther or pollen-specific genes and promoter sequences have been isolated, cloned and characterized, the development of transgenic male sterility systems has become possible. According to Kumar *et al.* (2000), these genes are expressed in either the gametophytic pollen or the sporophytic cells and tissues that either directly or indirectly support the development of pollen, such as the tapetum, filament, anther wall, etc. Nevertheless, the majority of crops lack the sterile source, which can be found by natural selection, synthetic mutation and protoplast fusion. Plant genetic engineering is developing quickly, providing a quick way to produce male sterile materials.

**Barnase-Barstar system (Abolition-restoration system):** In this approach, foreign trans-gene constructs interrupt (abolish; hence the term abolition) the process of pollen formation to produce transgenic male sterile plants. These transgenes typically encode cytotoxic substances that damage cellular integrity, such as lipase, protease and RNAase. Gametophytic and sporophytic cells are eliminated as a result of the expression of these genes, which is driven by a tissue-specific promoter in either the forming pollen or the tissues that support pollen development (typically tapetum cells). This leads to male sterility. Another trans-gene that blocks the effect of the disruptive gene is utilized to restore pollen fertility.

The first transgenic male sterility system, sometimes referred to as the Barnase-Barstar system and created by Mariani and his colleagues in tobacco and rapeseed, is a prime example of this type of system. The chimeric RNAase gene, known as Barnase, contains a tapetum-specific promoter (TA29), which was used to create transformed plants. Since the altered plants' tapetum cells produce the cytotoxic enzyme Barnase, the development of the tapetum cells and pollen was monitored, creating transgenic male sterile plants (Mariani *et al.,* 1990). However, in such male sterile plants, homozygosity of the Barnase gene cannot be attained and maintenance difficulties continue to be a hindrance. 50% hemizygous (Barnase -) male sterile F1s are produced when transgenic male sterile plants (hemizygous; Barnase -) are crossed with normal plants. These male sterile plants are unable to be used in crops where the fruits or seeds have a commercial value. It was shown that by producing F1s from the pollen of another transgenic plant with the Barstar gene combined with the TA29 promoter, the fertility of F1s produced from transgenic male sterile plants (dominant) could be recovered. The major suppressor of chimeric RNAase's cytotoxic products in F1 plants is the Barstar gene product. In tapetum cells, Barstar gene transcript formed complexes with chimeric RNAase transcribed from TA29-Barstar gene (Mariani *et al.,* 1992). Transgenic with a Barnase::herbicide resistance gene (linked) construct has been designed to address the maintenance issue. Because the herbicide resistance gene (HER2) is connected to the male sterility gene (Barnase), hemizygous male sterile plants can be bred with normal sister plants in these situations. Herbicide spraying on the progenies will ensure that 50% of the sterile segregants survive and 50% of the fertile segregants are eliminated.

|  |  |
| --- | --- |
| **Table 3: List of genetically engineered male sterile system** |  |
| **Gene for male sterility** | **Gene function** | **Promoter used** | **Salient features of male sterile plant** | **Source of gene** | **Plant** | **References**  |
| Barnase | Ribonuclease gene | TA29 | Tapetal cell layerdestruction, no pollenformation and restoredthrough barstar | *Bacillus amyloliquefaciens* | Tobacco, oilseed, rice,etc. | Kumar and Purty (2023);Mariani *et al*. (1990, 1992) |
| Cysteine protease | Protease | TA29 | Stamen length reduced, pollen was shrunken and deformed, Restored through Cystatin | *Arachis diogoi* | Tobacco, *Brassica* | Gautam *et al*. (2019);Shukla *et al*. (2014, 2016) |
| *BECLIN1* | Autophagy | TA29 and A9 | Tapetal degeneration, non-viable pollen, Conditional male sterile system | *Arabidopsis* | Tobacco | Singh *et al*. (2015) |
| *RIP* | Ribosome inactivatingprotein | TA29 | Tapetal tissue of the anther degenerated completely | *Dianthus chinensis* L | Tobacco | Cho *et al*. (2001) |
| *AtBI-1* | *Arabidopsis thaliana* ortholog of Baxinhibitor-1 | Osg6B promoter andLTP12 promoter | Tapetum degenerationand pollen abortion | *Arabidopsis* | Tobacco | Kawanabe *et al.* (2006) |
| *BoCysP1* and *BoCP3* | Cysteine proteasesinvolved in programmed cell death | A3 or A9 promoter | Anther were swollen andexcessively vacuolated | *Brassica oleracea* and*B. rapa* | *Arabidopsis* | *Konagaya et al.* (2008) |
| Cm-ETR1/H69A | Melon ethylene receptorgene | CaMV 35S (the cauliflower mosaic virus) | Abnormal stamen development | Melon | Tobacco | Takada *et al.* (2005) |
| *PbTM6a* and *PbTM6b* | B-class MADS-box | CaMV 35S | Decreased fertility of pollen grains | *Pyrus betulifolia* | Tomato | Zhang *et al*. (2023a, b) |
| MYC5-SRDX chimericrepressor | Transcription factor in JAhormone | MYC5 promoter andCaMV 35S | Defective stamen filament elongation | *Arabidopsis* | *Arabidopsis* | *Figueroa and Browse* (2015) |
| No Pollen 1 (*OsNP1*) | Glucose–methanol–choline oxidoreductase | CaMV 35S | Complete male sterility | *Oryza sativa* | Rice | Chang *et al*. (2016) |
| *BnaC.MAGL8.a* | Monoacylglycerol lipase | BnA9 promoter andCaMV35S promoter | Impaired pollen development | *Brassica napus* | *Arabidopsis* | *Gao et al. (2019)* |
| *PsEND1* | A pea anther-specific gene expressed inanther primordium | PsEND1 | Male sterility | *Pisum sativum* | *Arabidopsis*, Tobacco, | *Roque et al. (2019)* |

1. **Chemical Induction:**

(Source: Gautam *et al.,* 2023)

Chemicals or hormones are used to interfere with pollen development or function in the chemical induction of male sterility. Male gametocides and gibberellin biosynthesis inhibitors are two examples of chemicals that can specifically prevent pollen production, rendering male sterile. The substances known as chemical hybridizing agents (CHA) cause male sterility in plants. In 1950, it was discovered that some compounds, such as maleic hydrazide, might cause selected male sterility in maize (Moore, 1950; Naylor, 1950). It was acknowledged that there might be some benefits despite certain drawbacks, particularly in the time needed to find commercially feasible hybrids. This is because chemical means of inducing male sterility can eliminate the need for the protracted process needed to produce male sterile and restoration lines.

**Site and mode of action of CHA:** The most significant general characteristic that the literatures have shown is that the previously discovered compounds (e.g., FW-450, ethephon, RH-531, PPX 3778) can cause a variety of particular effects that vary according to the interaction between treatment time and dosage. Among the general impacts are the following: (McRae, 1985): (A) Early disruption of meiosis and early stoppage of the next developmental stage. (b) Microspores have thin walls, a deformed shape and are not viable due to disruptions in exine production. (c) Abnormalities in the microspore vacuoles, reduced starch deposition and ongoing tapetum. (d) While anthers are normal, pollen is not viable. Although there is pollen and it is viable, anthers either do not dehisce at all or do so slowly.

**Mechanisms of male sterility**

**(1) Cytological changes:** The microsporogenesis process's pre- and post-meiotic stages could ultimately break down. The abnormalities may include aberration at the mature or nearly mature pollen stage, at the vacuolate microspore stage, during the meiotic process in the development of tetrads, or at the release of tetrad (the dissolution of callose).

**(2) Biochemical changes:** A few biochemical alterations, including changes in the structure and amount of proteins, amino acids and anther-developing enzymes, are linked to male sterility. It has been discovered to be connected to elevated amounts of aspartic acid, glycine and arginine and decreased levels of proline, leucine, isoleucine, phenylalanine and valine (Kaul, 1988). It has been discovered that proline levels are especially impacted. According to Kakihara et al. (1988), mature male sterile anthers had one-eighth the proline content of fertile anthers. Less polypeptide bands and less soluble protein can be found in the anthers of male sterile plants. Mutant stamens lacked certain polypeptides that were synthesized in normal stamens.

1. **Cytoplasmic Male Sterility (CMS):**

CMS is the outcome of interacting nuclear and cytoplasmic genomes, which stops pollen formation. CMS is frequently linked to chimeric open reading frames being expressed in the mitochondrial genome and rearrangements of mitochondrial DNA (Rahman *et al.,* 2024). Since the mt gene expresses male sterility and the mitochondria are typically removed from the pollen after fertilization, cytoplasmic male sterility is a characteristic that mothers inherit. Since a zygote's cytoplasm predominantly originates from an egg cell, such male-sterile plants would always produce male-sterile offspring. By employing a particular strain as a pollinator (recurrent parent) in the backcross program's subsequent generations, CMS can be readily transferred to that strain. The male sterile line's nuclear genotype would be nearly identical to the recurrent pollinator strain after six to seven backcrosses.

1. **Environmental Manipulation:**

Pollen development and fertility can be influenced by environmental conditions, including temperature, photoperiod and nutrition availability. Environment Sensitive Genetic Male Sterility (EGMS) is the term used to describe the induction of male sterility in crops through the manipulation of environmental circumstances at critical stages of pollen production.

Some genetic lines of male sterility are conditional mutants, meaning that male sterility only manifests itself under a specific set of environmental conditions, the lack of which causes the male sterile plants to become male fertile. These GMS mutants are categorized as Temperature sensitive Genic Male Sterile (TGMS) lines or Photoperiod sensitive Genic Male Sterile (TGMS) lines once the crucial environment typically temperature or photoperiod for sterility and fertility expression is determined. Temperature-sensitive EGMS lines have been observed in several vegetable crops, including tomato, carrot, cabbage, Brussels sprouts, broccoli and peppers (sweet pepper and Chilli). However, the majority of these were previously recognized as typical genic male sterile lines. (Kumar *et al.,* 2000).

EGMS lines were once thought to be extremely impractical because due to the instability issue, yet they currently stand as the most effective approach for producing hybrid seeds. Practically speaking, nevertheless, it is important to determine the critical temperature or photoperiod for the manifestation of fertility/sterility in temperature- and photoperiod-sensitive genetic male sterility, respectively.

1. **Epigenetic Regulation:**

Pollen development-related gene expression patterns can be regulated by epigenetic alterations such DNA methylation and histone modifications (Wan *et al.,* 2021). Choosing targets at epigenetic regulators provides a viable approach to causing male sterility in crops.

1. **Utilization of marker gene:**

One recessive gene that is expressed in the seedling stage and governs the non-lobbing leaf characteristic of water melon can be employed as a marker gene to facilitate the simple and cost-effective generation of hybrid seed (Whitaker and Davis, 1962). Seeds from the non-lobed lines are the only ones that can be gathered. The inbred lines of lobed and non-lobed can be sown in alternating rows. When they are still seedlings, it is easy to identify the F1 hybrids with lobed leaves. However, since only approximately one-third of the seedlings will be F1 hybrids on average, roughly 6–8 seeds/hill may be sowed.

Swarup and Gill (1964) proposed using a marker gene in cabbage for purple stem pigmentation to make it easier to identify F1 hybrid seedlings before transplanting. To produce F1 hybrid seeds, brussels sprouts is crossed with a recessive marker gene for glossy foliage (North and Priestley, 1962). Johnson (1966) recommended adding a recessive marker gene to the Brussels sprout A and B lines and proposed a partial chlorosis trait for this reason in addition to the glossy foliage.

Using an inbred line with brown seeds, Davis (1966) proposed a novel technique for creating hybrid onions, demonstrating how the hue of the brown seed coat is associated with male sterility. One recessive gene controls the hue of the brown seed coat. Hybrid seeds can be produced from the black-seeded pollen parent line and the brown-seeded male sterile line. Black hybrid seeds will be extracted from the male sterile line. This procedure is also helpful in rouging off kinds seen in male fertile pollen parents and male sterile seed parents.

**Conversion Breeding and Principles of Introducing Male Sterility Traits**

A conversion breeding technique aims at inducing desirable characteristics, such as male sterility, in elite germplasm or cultivars. To restore the genetic heritage of the recurrent parent and introgress the desired trait, this method usually entails several rounds of backcrossing and selection (Xu *et al.,* 1997). Male sterility is an essential characteristic in the generation of hybrid seeds, which makes it possible to produce high-yielding hybrid cultivars with better uniformity and vigour.

Introducing male sterility traits into breeding lines involves several key principles to ensure successful conversion and maintain agronomic performance:

* **Identification of Male Sterility Sources:** Identifying and characterizing male sterile lines or sources with stable and heritable male sterility traits is the first step in conversion breeding.
* **Marker-Assisted Selection (MAS):** Effective selection and introgression of male sterility features during backcrossing are made possible by the use of molecular markers associated with male sterility genes. Photoperiod-sensitive male sterile japonica rice with great cross-compatibility with indica rice bred using marker assistance (Liangming *et al.,* 2010). The limited fertility of rice hybrids between Japonica and Indica has prevented breeders from taking advantage of the significant heterotic potential of these hybrids. Simple introgression at the main sterility loci can overcome hybrid sterility brought on by an allelic interaction at a few loci. They introduce a photoperiod-sensitive male sterility gene from cv. Lunhui 422S (*indica*) and the yellow leaf gene from line Yellow249 (*indica*) into the elite japonica cv. Zhendao 88 by marker-assisted backcrossing. The fertility genes S5, S8, S7 and S9 were tagged using the microsatellite markers RM276, RM455, RM141 and RM185, in that order. The male sterile plant Line 509S is photoperiod-sensitive and true-breeding; its morphology is similar to that of the japonica type. According to genotypic research, 92% of the DNA in line 509S is from Japan. Although line 509S is cross-compatible with indica types and the resulting hybrids express a large degree of heterosis, hybrids between japonica kinds and the line experience hybrid sterility. All of these findings point to the segment replacement on fertility loci based on information already available as well as marker-assisted selection as a useful strategy for making use of the inter-subspecies heterosis in rice.
* **Backcrossing and Recurrent Selection:** Backcrossing to the recurrent parent is essential to recover its genetic background while incorporating male sterility traits. Recurrent selection further enhances converted lines' performance through iterative selection and recombination cycles.
* **Genetic Mapping and Gene Discovery:** Genetic mapping and gene discovery efforts help elucidate the genetic basis of male sterility and identify candidate genes or loci for targeted introgression. Fine mapping of a male sterility gene *ms-3* in cucumber (Han *et al.,* 2018) and gene *MS-cd1* in Brassica oleracea (Zhang *et al.,* 2011).
* **Evaluation of Agronomic Performance:** Converted lines must undergo precise evaluation for agronomic performance, including yield potential, stress tolerance and quality traits, to ensure that male sterility introduction does not compromise overall performance.
* **Hybrid Seed Production:** Male sterile lines are utilized in hybrid seed production to exploit hybrid vigor, resulting in increased yield and uniformity in commercial crops.

These principles guide the successful introduction of male sterility traits through conversion breeding, facilitating the development of hybrid cultivars with improved performance and productivity.

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